



RECEIVED
OPPT CBIC

05 MAR -7 AM 10:48

One Houston Center, Suite 1600
1221 McKinney Street
Houston, Texas 77010
Phone: 713.309.2136
Fax: 713.951.1574
Patrick.gibson@lyondell.com

March 4, 2005

Document Processing Center (7407M)
EPA EAST - Room 6428
Office of Pollution Prevention and Toxics
United States Environmental Protection Agency
1201 Constitution Avenue, NW
Washington DC 20002

8EHQ-0305-15943

Attention: TSCA 8(e) Coordinator

RE: Allyl Alcohol - Algal Toxicity Study

CONTAINS NO CBI

Dear Sir or Madam:

RECEIVED
OPPT NCIC
2005 MAR 14 PM 1:22

Lyondell Chemical Company (Lyondell) hereby submits this letter pursuant to Section 8(e) of the Toxic Substances Control Act (TSCA) and EPA's 1991 Section 8(e) Reporting Guide because it includes findings that EPA may consider reportable. In an algal toxicity study, Allyl alcohol (CASRN 107-18-6) was found to be toxic to the unicellular green alga, *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*):

Hour	EC Type	EC Value (mg/L)	95% Confidence Limits (mg/L)	NOEC (mg/L)
72	E _b C ₅₀	2.25	2.21 and 2.30	0.930
72	E _r C ₅₀	5.38	5.28 and 5.47	0.930

While these toxic effects were expected, it is the first time that they have been reported for an algal species. Lyondell has not made a determination as to whether a significant risk of injury to health or the environment is actually presented by these findings.

A copy of the study final report is enclosed for your review.

Allyl alcohol was tested pursuant to Lyondell's testing plan for the High Production Volume Chemical Challenge

Should you have any questions or require additional details, please do not hesitate to call me at 713-309-2136. I may also be reached by by e-mail at patrick.gibson@lyondell.com.

Sincerely,

Patrick Gibson
Senior Product Safety Specialist - Regulatory
Corporate TSCA Coordinator
Lyondell Chemical Company



Enclosure


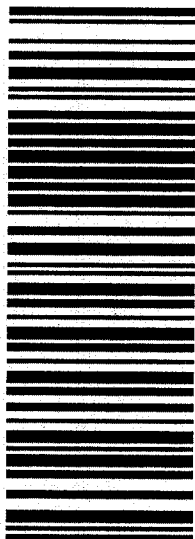
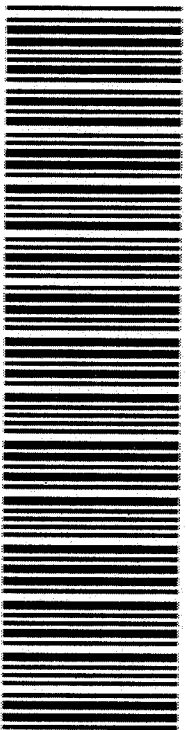
Cc: TSCA 8(e) Files



MR284159

Lyondell Chemical Company



		EXP		Parcels: 1/1
From: ALYONDELL EQUISTAR P. Gibson 1221 MCKINNEY ST HOUSTON, TX 77010 UNITED STATES			ORIGIN: GLS Sender's ref	
To: US Environmental Protection Agency TSCA Section 8(e) Coordinator 1201 Constitution Avenue, NW EPA East MailMail Code 7407M Washington, DC 20004 UNITED STATES			POSTCODE: 20004	
Tel: 202-564-4780				
Description: TSCA 8(e) Submissions - AA				
Weight: Letter				
Date: 2005-03-04				
DHL standard terms and conditions apply.				
 DCAA 4X (2L)US20004				
 WABILL: 25270505455 (Non-Negotiable)				

Please fold or cut in half

DO NOT PHOTOCOPY

Using a photocopy could delay the delivery of your package and will result in additional shipping charge

For Tracking, please go to www.dhl-usa.com or call 1-800-225-5345

Thank you for shipping with DHL

[Create New Shipment](#)[View Pending Shipments](#)

STUDY TITLE

Toxicity of Allyl Alcohol 20906MB (Lyondell lot number CX30609214) to the
Unicellular Green Alga, *Pseudokirchneriella subcapitata*

DATA REQUIREMENT

OECD Guideline 201

AUTHOR

Stephen L. Hicks

STUDY INITIATION DATE

June 8, 2004

STUDY COMPLETION DATE

February 18, 2005

SPONSOR

Lyondell Chemical Company
One Houston Center, Suite 1600
1221 McKinney
Houston, Texas 77010

LABORATORY PERFORMING BIOLOGICAL PHASE

ABC Laboratories, Inc.
7200 E. ABC Lane
Columbia, Missouri 65202

LABORATORY PERFORMING ANALYTICAL PHASE

Environmental Chemistry, Inc.
2525 West Bellfort, Suite 175
Houston, Texas 77054

PROJECT IDENTIFICATION

ABC Study No. 48910

STATEMENT OF GLP COMPLIANCE

Compliance Statement for ABC Study No. 48910 entitled, "Toxicity of Allyl Alcohol 20906MB (Lyondell lot number CX30609214) to the Unicellular Green Alga, *Pseudokirchneriella subcapitata*," for Lyondell Chemical Company.

The Study Director for the above-referenced test herein confirms that the study was conducted in compliance with the OECD Principles of Good Laboratory Practice, Revised Principles of GLP [C(97)186/Final], with the following exceptions: 1) test substance characterization was not conducted in accordance with the Principles of Good Laboratory Practice and 2) analytical confirmation of the test substance concentrations was not conducted in accordance with the Principles of Good Laboratory Practice.

All original raw data were submitted to the Sponsor along with the final report. A copy of the final report, copies of all raw data from the study, and all original facility records have been archived at ABC Laboratories.

Stephen L. Hicks

Stephen L. Hicks
Study Director
ABC Laboratories, Inc.

18 Feb 05

Date

QUALITY ASSURANCE STATEMENT

ABC's Quality Assurance Unit reviewed ABC Study No. 48910 entitled, "Toxicity of Allyl Alcohol 20906MB (Lyondell lot number CX30609214) to the Unicellular Green Alga, *Pseudokirchneriella subcapitata*," for Lyondell Chemical Company. The following audits/inspections were conducted on this study.

Date of Study-Based Inspection	Phase Inspected	Date Reported to the Study Director	Date Reported to ABC Management
29 OCT 04	Procedure: Day 3 Cell Counts	29 OCT 04	30 OCT 04
27 JAN 05	Draft/Final Report and Raw Data	27 JAN 05	16 FEB 05

These audits indicate that the report is an accurate reflection of the study as it was conducted by ABC Laboratories, Inc.

Jessica Dutton
Jessica Dutton
Quality Assurance Officer II

18 Feb 05
Date

STUDY PERSONNEL

NAME

TITLE

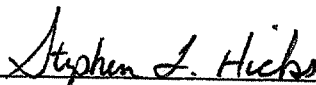
Stephen L. Hicks
James Herrick

Research Scientist
Associate Technician

SIGNATURE PAGE

Submitted by: ABC Laboratories, Inc.
7200 E. ABC Lane
Columbia, Missouri 65202

Prepared by:




Stephen L. Hicks
Research Scientist
ABC Laboratories, Inc.

18 Feb 05

Date

Approved by:



Jon E. Rhodes, M.S.
Director, ABC Chemical Services
ABC Laboratories, Inc.

18 Feb 05

Date

TABLE OF CONTENTS

	<u>Page No.</u>
TITLE PAGE.....	1
STATEMENT OF GLP COMPLIANCE.....	2
QUALITY ASSURANCE STATEMENT.....	3
STUDY PERSONNEL.....	4
SIGNATURE PAGE.....	5
TABLE OF CONTENTS.....	6
COMPENDIUM.....	8
1.0 INTRODUCTION.....	9
2.0 MATERIALS AND METHODS.....	9
2.1 Test Substance.....	9
2.2 Test Organism.....	9
2.3 Test Medium.....	9
2.4 Test Methods.....	10
2.4.1 Range-Finding Test.....	10
2.4.2 Definitive Test.....	10
2.5 Analytical Confirmation.....	11
2.6 Statistical Analysis.....	11
2.6.1 NOEC Estimates.....	11
2.6.2 Percent Inhibition Calculations and EC Estimates.....	12
3.0 RESULTS AND DISCUSSION.....	13
3.1 Analytical Results.....	13
3.2 Biological Results.....	13
3.3 Water Quality.....	14
4.0 CONCLUSIONS.....	15
REFERENCES.....	16
Table 1. Measured Concentrations of Allyl Alcohol in Test Solutions During the 72-Hour Toxicity Test with the Unicellular Green Alga, <i>Pseudokirchneriella subcapitata</i>	17
Table 2. Mean Cell Density of the Green Alga, <i>Pseudokirchneriella subcapitata</i> , Exposed to Allyl Alcohol 20906MB.....	18

Table 3.	Replicate Cell Density for the Green Alga, <i>Pseudokirchneriella subcapitata</i> , During a 72-Hour Exposure to Allyl Alcohol 20906MB	19
Table 4.	Mean Area Under the Growth Curve Values for the Green Alga, <i>Pseudokirchneriella subcapitata</i> , Exposed to Allyl Alcohol 20906MB	20
Table 5.	Mean Growth Rate Values for the Green Alga, <i>Pseudokirchneriella subcapitata</i> , Exposed to Allyl Alcohol 20906MB	21
Table 6.	Calculated EC Values Based on Nominal Concentrations of Allyl Alcohol 20906MB During an Exposure of the Green Alga, <i>Pseudokirchneriella subcapitata</i>	22
Table 7.	Calculated EC Values Based on the Adjusted Geometric Mean of the Measured Concentrations of Allyl Alcohol 20906MB During an Exposure of the Green Alga, <i>Pseudokirchneriella subcapitata</i>	23
Table 8.	Temperature and pH Measurements During the Exposure of <i>Pseudokirchneriella subcapitata</i> to Allyl Alcohol 20906MB.....	24
Figure 1.	Growth Curves for the Green Alga, <i>Pseudokirchneriella subcapitata</i> , During a 72-Hour Exposure to Allyl Alcohol 20906MB	25
APPENDIX A. ABC REAGENT WATER CHARACTERIZATION		26
APPENDIX B. PROTOCOL, AMENDMENTS, AND DEVIATION		28
APPENDIX C. ANALYTICAL DATA		45
APPENDIX D. REPLICATE AREA UNDER THE GROWTH CURVE.....		55
APPENDIX E. REPLICATE GROWTH RATE.....		57

COMPENDIUM

Study Title: Toxicity of Allyl Alcohol 20906MB (Lyondell lot number CX30609214) to the Unicellular Green Alga, *Pseudokirchneriella subcapitata*

Test Substance: Allyl Alcohol 20906MB (Lyondell lot number CX30609214)

Test Medium: Freshwater Algal Nutrient Medium at pH 7.5 ± 0.1

Experimental Dates: Start – October 26, 2004 (definitive test initiation)
Termination – November 18, 2004 (analytical confirmation completed)

Length of Study: 72 hours

Temperature Range of Test Solutions: 22.5 to 24.0°C

pH Range of Test Solutions: 7.5 to 9.2

Nominal Test Concentrations: 0 (control), 0.65, 1.3, 2.5, 5.0, and 10 mg total product/L

Geometric Mean Measured Concentrations (adjusted for analytical recovery): <0.040 (control), 0.343, 0.930, 2.41, 6.03, and 9.12 mg total product/L

Results Based on Nominal Concentrations:

Hour	EC Type	EC Value (mg/L)	95% Confidence Limits (mg/L)	NOEC (mg/L)
72	E _b C ₅₀	2.4	2.3 and 2.4	1.3
	E _r C ₅₀	3.8	3.5 and 4.0	1.3

Results Based on the Geometric Mean of Measured Concentrations (adjusted for analytical recovery):

Hour	EC Type	EC Value (mg/L)	95% Confidence Limits (mg/L)	NOEC (mg/L)
72	E _b C ₅₀	2.25	2.21 and 2.30	0.930
	E _r C ₅₀	5.38	5.28 and 5.47	0.930

1.0 INTRODUCTION

ABC Laboratories, Inc. was contracted to conduct a 72-hour toxicity test with *Pseudokirchneriella subcapitata* exposed to Allyl Alcohol 20906MB for Lyondell Chemical Company. The criterion for effect was inhibition in cell growth (measured as biomass or growth rate), relative to the control. Results of the test are expressed as E_bC_{50} and E_rC_{50} concentrations of Allyl Alcohol 20906MB calculated to reduce biomass and growth rate, respectively, by 50 percent at the specified time.

2.0 MATERIALS AND METHODS

2.1 Test Substance

A sample of Allyl Alcohol 20906MB (Lyondell lot number CX30609214) was received from Sigma-Aldrich on June 1, 2004, and was assigned ABC reference no. TS-16534. The sample was stored at room temperature. The sample was used to prepare exposure solutions during the range-finding and definitive tests. The following test substance information was received from the Sponsor:

Form:	Colorless liquid
Purity:	99.38%
Expiration date:	Not provided

2.2 Test Organism

The parent stock of *Pseudokirchneriella subcapitata*, formally known as *Selenastrum capricornutum*, was obtained from the Department of Botany, Culture Collection of Algae, University of Texas at Austin, on April 6, 2004. The parent stock was identified as *Selenastrum capricornutum*. The prepared cultures were maintained in a temperature-controlled environmental chamber under continuous light. Periodically, new cultures were cloned from an existing culture derived from the parent stock. All cultures were maintained under the same conditions as those used for testing. The algal culture used for this test was four days old at test initiation.

2.3 Test Medium

The test medium was a freshwater algal nutrient medium (2). The medium was prepared by the addition of appropriate reagent grade salts to autoclaved ABC reagent water. ABC reagent water is produced by passing reverse-osmosis water through a series of deionization tanks, a laboratory water purification system consisting of carbon, de-mineralization, and organic adsorption cartridges, and then through a 0.2- μ m filter. After preparation, the medium, at pH 7.5 ± 0.1 , was filtered through Millipore® 0.45- μ m filters. Chemical characterization of a representative sample of ABC reagent water is presented in Appendix A.

2.4 Test Methods

Test procedures followed the ABC test protocol entitled, "Toxicity of Allyl Alcohol 20906MB (Lyondell lot number CX30609214) to the Unicellular Green Alga, *Pseudokirchneriella subcapitata*," and amendments (Appendix B). This protocol was designed to comply with Organization of Economic Cooperation and Development method 201 (3).

2.4.1 Range-Finding Test

A range-finding test was performed from June 14 to 17, 2004, at nominal test concentrations of 0 (control), 0.10, 1.0, 10, and 100 mg total product/L. After 72 hours of exposure, the percent change in cell growth as compared to control was +38% in the 0.10 mg total product/L treatment, +4% in the 1.0 mg total product/L treatment, -98% in the 10 mg total product/L treatment, and -98% in the 100 mg total product/L treatment. All test solutions appeared clear with no color associated with the test substance, and with no visible precipitates, surface films, or undissolved test substance. Based on these results, nominal concentrations of 0 (control), 0.65, 1.3, 2.5, 5.0, and 10 mg total product/L were selected for the definitive test.

2.4.2 Definitive Test

The in-life phase of the definitive test was conducted from October 26 to 29, 2004. A 0.010 mg/mL primary standard was prepared by diluting 0.0102 g of Allyl Alcohol 20906MB to a 1,000 mL volume with test medium. The primary standard was used at the highest treatment and the four lower treatments were prepared individually by diluting appropriate volumes of the primary standard with test medium. One hundred-milliliter volumes of the resulting solutions were transferred to the exposure flasks.

The exposure flasks were 250-mL Erlenmeyer flasks with foam stoppers and labeled with study number, treatment, replicate, and grid position. Prior to test initiation, the flasks were cleaned and autoclaved according to ABC standard operating procedures. Each treatment was replicated three times (replicates A, B, and C) and each replicate contained 100 mL of the appropriate parent solution. An additional replicate (replicate D) of the lowest test substance treatment, containing 100 mL of the appropriate parent solution, was also prepared and used to evaluate incorporation of the test substance into the algal biomass. At test initiation, each A, B, and C replicate was inoculated with 1.0 mL of an algal concentrate containing approximately 1.0×10^6 cells/mL, resulting in a final density of approximately 1.0×10^4 cells/mL for each flask. At 24, 48, and 72 hours, cell density was measured in all replicates of each treatment by direct microscopic counting with a hemacytometer. All cell density measurements, with the exception of the 72 hour cell density measurements, were performed ± 1 hour from test initiation. The cell density measurements at 72 hour were performed 20 minutes prior to the observation point required by the protocol, and this deviation did not affect the integrity of the study. Replicate D of the lowest test substance treatment was not inoculated with algae.

During the three-day exposure period, the flasks were randomly positioned using a computer generated random number table and incubated at $24 \pm 2^\circ\text{C}$ in a temperature controlled environmental chamber under continuous cool-white fluorescent lighting. A continuous recording of environmental chamber temperature was made from one uninoculated blank flask using an electronic datalogger with thermistor probe. Light intensity was measured daily with a

LI-COR Model LI-189 light meter equipped with a LI-COR photometric sensor and ranged from 8,561 to 8,679 lux. The flasks were swirled on an orbital shaker table at approximately 100 rpm throughout the test. Temperature and pH were measured in all parent solutions prior to distribution of the solutions to the test flasks. At 72 hours, temperature and pH were measured in replicate A of all treatments. Temperature and pH were measured with a WTW pH 330i meter.

2.5 Analytical Confirmation

Samples were collected from the control and each test substance treatment at 0 and 72 hours of the definitive test. The 0 hour samples were collected from the parent solutions. The 72 hour samples were collected from pooled solutions after combining replicate solutions (replicates A, B, and C) by treatment. At 72 hours, a sample from the 0.65 mg total product/L abiotic treatment was collected directly from the test flask. Sampling began with the control and continued up to the highest test substance treatment. Each sample volume was approximately 50 mL. Each sample was acidified to a pH <2.0 with HCl then transferred into an appropriately labeled clear, glass, 40-mL vial, filling the vial completely, and sealing the vial with no-headspace.

All samples collected were shipped November 9, 2004, via next day delivery to Environmental Chemistry, Inc. for analysis. Each sample was stored refrigerated prior to shipment. The samples were analyzed in accordance with EPA Method 8620 using GC/MS. Sample introduction into the GC/MS was accomplished using the heated purge and trap Method 5030.

The geometric mean of the measured concentrations, as presented in this report, was calculated using the following equation:

$$GM = \text{Antilog} \frac{[\text{Log}R1 + \text{Log}R2]}{2}$$

where:

GM	=	geometric mean
R1	=	measured concentration at time 0
R2	=	Measured concentration at termination

2.6 Statistical Analysis

2.6.1 NOEC Estimates

The NOECs, based on cell density, area under the growth curve, and growth rate, were estimated using a one-way analysis of variance (ANOVA) procedure and a two-tailed Dunnett's test. The alternate hypothesis was the mean for the growth parameter was reduced or enhanced in comparison to the pooled control mean. Prior to the Dunnett's test, a Shapiro-Wilk's test and a Levene's test were conducted to test for normality and homogeneity of variance, respectively, over treatments at each time point. If the results from the Shapiro-Wilk's and Levene's tests indicated normality and insignificant heterogeneity, the analysis was performed on the non-transformed raw data. In instances of non-normality or heterogeneity, a square root transformation was performed. If both the non-transformed raw data and the transformed data

exhibited non-normality or inequality of variance, a non-parametric analysis of variance was performed on the ranks of the raw data values. Non-parametric analyses were performed on the 48- and 72-hour growth rate data. Parametric analyses were performed on the 24-, 48-, and 72-hour area under the growth curve data and the 24-hour growth rate data.

2.6.2 Percent Inhibition Calculations and EC Estimates

The area under the growth curve in each treatment was calculated from 0-hour to 24, 48, and 72 hours using the following equation:

$$A = \frac{N_1 - N_0}{2} \times t_1 + \frac{N_1 + N_2 - 2N_0}{2} \times [t_2 - t_1] + \dots + \frac{N_{n-1} + N_n - 2N_0}{2} \times [t_n - t_{n-1}]$$

where:

A	=	area
N _n	=	cell density at n th measurement from start, n=1,2,...n
N ₀	=	cell density at time 0
t _n	=	time of n th measurement (hours after start), n=1,2,...n

The growth rate in each treatment was calculated for each period, i.e., 0 to 24, 0 to 48, and 0 to 72 hours using the following equation:

$$\mu = \frac{\ln N_n - \ln N_1}{t_n - t_1}$$

where:

μ	=	growth rate
N _n	=	cell density at second time point
N ₁	=	cell density at time 0
t _n	=	second time point
t ₁	=	time 0

E_bC₅₀, and E_rC₅₀ estimates were calculated using a logistic (sigmoid-shaped) model fit to the data with percent inhibition as the dependent variable and concentration as the independent variable. The percent inhibition was calculated based on area under the growth curve and growth rate. The model used to describe the response to increasing test substance concentration was the four-parameter logistic model with two parameters fixed; the minimum percent inhibition (A) at 0%, and the maximum percent inhibition (D) at 100%. The model was fit only in instances where the mean percent inhibition at the highest test substance treatment was greater than 45%. The model is:

$$\%I = D + \left[\frac{(A - D)}{(1 + \text{CONC}^B) (EC_{50}^{-B})} \right]$$

where:

I = inhibition

CONC = test concentration

B = slope

EC₅₀ = concentration corresponding to 50% inhibition

The distribution of x hat method was used to estimate the 95% confidence limits.

3.0 RESULTS AND DISCUSSION

3.1 Analytical Results

Analytical results, presented as directly measured values, means over the test period, adjusted means (corrected for mean recovery of Allyl Alcohol from spiked samples: 110.7%), and geometric means (uncorrected and corrected for mean recovery of Allyl Alcohol from spiked samples: 110.7%) are given in Table 1. The adjusted geometric mean concentrations of Allyl Alcohol (considered to give the best representation of actual test concentrations) were 0.343, 0.930, 2.41, 6.03, and 9.12 mg total product/L, representing 53, 72, 96, 120, and 91%, respectively, of the nominal concentrations (Table 1). No residues of Allyl Alcohol above the sample quantitation limit of 0.040 mg/L were detected in the control. The 72 hour measured concentrations of Allyl Alcohol in the biotic (inoculated) and abiotic (uninoculated) replicates established at a nominal 0.65 mg/L were closely similar (0.341 and 0.348 mg/L; Table 1), indicating little or no adsorption of the test material onto algal cells. Analytical data is presented in full in Appendix C. All test solutions appeared clear with no color associated with the test substance, and with no visible precipitates, surface films, or undissolved test substance.

3.2 Biological Results

After 72 hours of exposure, the mean cell density in the control was 118×10^4 cells/mL (Table 2). This value represented an increase of 118 times the initial target inoculation density and demonstrated control growth was acceptable for the test. The mean cell density at 72 hours ranged from 1.0×10^4 cells/mL in the 10 mg total product/L treatment to 124×10^4 cells/mL in the 0.65 mg total product/L treatment (Table 2). Percent difference in cell density, as compared to the control, ranged from -99% in the 10 mg total product/L treatment to +5% in the 0.65 mg total product/L treatment (Table 2). Cell counts for all replicates are presented in Table 3. Growth curves for the control and all Allyl Alcohol treatments are presented in Figure 1.

The mean area under the growth curve values for the control and all test substance treatments are presented in Table 4. Area under the growth curve values for individual replicate flasks are provided in Appendix D. Percent difference in area under the growth curve, as compared to the control, ranged from -100% at 10 mg total product/L to +5% at 0.65 mg total product/L after 72 hours of exposure to Allyl Alcohol (Table 4). One-way analysis of variance showed a significant ($p = 0.05$) reduction in the area under the growth curve for treatments ≥ 2.5 mg total product/L as compared to the control at 72 hours. The NOEC at 72 hours was 1.3 mg total product/L, based on the lack of a statistically significant reduction in area under the growth curve

at this and lower test substance treatments. Based on nominal concentrations and area under the growth curve, the E_bC_{50} at 72 hours was 2.4 mg total product/L (95% confidence limits: 2.3 and 2.4 mg total product/L). Based on the adjusted geometric mean of the measured concentrations and area under the growth curve, the E_bC_{50} at 72 hours was 2.25 mg/L (95% confidence limits: 2.21 and 2.30 mg/L). All results of the statistical evaluations with area under the growth curve are presented in Table 6 (nominal concentrations) and Table 7 (adjusted geometric mean of the measured concentrations).

The mean growth rate values for the control and all test substance treatments are presented in Table 5. Growth rate values for individual replicate flasks are provided in Appendix E. Percent difference in the growth rate, as compared to the control, ranged from -100% at 10 mg total product/L to +2% at 0.65 mg total product/L after 72 hours of exposure to Allyl Alcohol (Table 5). One-way analysis of variance showed a significant ($p = 0.05$) reduction in the growth rate for treatments ≥ 2.5 mg total product/L as compared to the control at 72 hours. The NOEC at 72 hours was 1.3 mg total product/L, based on the lack of a statistically significant reduction in the growth rate at this and lower test substance treatments. Based on nominal concentrations and growth rate, the E_rC_{50} at 72 hours was 3.8 mg total product/L (95% confidence limits: 3.5 and 4.0 mg total product/L). Based on the adjusted geometric mean of the measured concentrations and growth rate, the E_rC_{50} at 72 hours was 5.38 mg/L (95% confidence limits: 5.28 and 5.47 mg/L). All results of the statistical evaluations with growth rate are presented in Table 6 (nominal concentrations) and Table 7 (adjusted geometric mean of the measured concentrations).

3.3 Water Quality

Test solution temperature ranged from 22.5 to 24.0°C at 0 and 72 hours (Table 8). The temperature data from the datalogger indicated that the temperature of the environmental chamber during the definitive test remained within the $24 \pm 2^\circ\text{C}$ range specified in the protocol. Test solution pH at test initiation was 7.5 for the control and all test substance treatments (Table 8). Test solution pH at 72 hours ranged from 7.7 to 9.2 (Table 8). The pH of the control and test substance treatments ≤ 1.3 mg total product/L at 72 hours deviated more than 1 pH unit from the initial pH as a result of the algal biomass present at 72 hours. The pH deviation of more than 1 pH unit did not affect the integrity of the test since acceptable growth ($>16\text{X}$ increase) was observed in the control.

4.0 CONCLUSIONS

Results based on nominal concentrations:

Hour	EC Type	EC Value (mg total product/L)	95% Confidence Limits (mg total product/L)	NOEC (mg total product/L)
24	E _b C ₅₀	2.2	2.0 and 2.3	0.65
	E _r C ₅₀	2.3	2.0 and 2.7	1.3
48	E _b C ₅₀	2.2	2.1 and 2.4	1.3
	E _r C ₅₀	3.3	2.7 and 3.8	1.3
72	E _b C ₅₀	2.4	2.3 and 2.4	1.3
	E _r C ₅₀	3.8	3.5 and 4.0	1.3

Results based on the geometric mean of the measured concentrations (adjusted for analytical recovery):

Hour	EC Type	EC Value (mg/L)	95% Confidence Limits (mg/L)	NOEC ^a (mg/L)
24	E _b C ₅₀	2.09	1.95 and 2.23	0.343
	E _r C ₅₀	2.26	1.90 and 2.61	0.930
48	E _b C ₅₀	2.11	1.77 and 2.46	0.930
	E _r C ₅₀	5.14	4.79 and 5.50	0.930
72	E _b C ₅₀	2.25	2.21 and 2.30	0.930
	E _r C ₅₀	5.38	5.28 and 5.47	0.930

REFERENCES

- (1) Organization for Economic Cooperation and Development. 1997. Decision of the Council, Revised Principles of GLP [C(97)186/Final].
- (2) American Society for Testing and Materials (ASTM). 1997. Standard Guide for Conducting Static 96-h Toxicity Tests with Microalgae. ASTM Designation E1218-97a.
- (3) Organization for Economic Cooperation and Development (OECD). June 7, 1984. OECD Guidelines for Testing of Chemicals. Algae, Growth Inhibition Test, OECD Guideline No. 201.

Table 1. Measured Concentrations of Allyl Alcohol in Test Solutions During the 72-Hour Toxicity Test with the Unicellular Green Alga, *Pseudokirchneriella subcapitata*

Nominal Concentration (mg total product/L)	Measured Concentration (Percent Nominal)					Adjusted Geometric Mean ^a (mg/L)
	0 Hr (mg/L)	72 Hr (mg/L)	Mean 0-72 Hr (mg/L)	Adjusted Mean ^a (mg/L)	Geometric Mean (mg/L)	
Control	<SQL ^b	<SQL ^b	---	---	---	---
0.65	0.424 (65)	0.341 (52)	0.383 (59)	0.346 (53)	0.380 (58)	0.343 (53)
0.65 (abiotic)	---	0.348 (54)	NA	NA	NA	NA
1.3	1.32 (102)	0.803 (62)	1.06 (82)	0.958 (74)	1.03 (79)	0.930 (72)
2.5	3.75 (150)	1.90 (76)	2.83 (113)	2.56 (102)	2.67 (107)	2.41 (96)
5.0	9.00 (180)	4.95 (99)	6.98 (140)	6.31 (126)	6.67 (133)	6.03 (120)
10	12.8 (128)	7.91 (79)	10.4 (104)	9.39 (94)	10.1 (101)	9.12 (91)

^a Results corrected for mean Allyl Alcohol recovery as determined in duplicate spiked samples of test medium (81.4 and 140.0%, mean = 110.7%). These matrix recovery values are applicable to the tabulated results for test samples, but other matrix recovery samples showed a range of 122 – 204% indicating a degree of imprecision in the quantitative analyses (perhaps associated with variation in Allyl Alcohol retention during sample processing).

^b Sample Quantitation Limit (SQL) = 0.040 mg/L

NA = Not applicable.

Table 2. Mean Cell Density of the Green Alga, *Pseudokirchneriella subcapitata*, Exposed to Allyl Alcohol 20906MB

Nominal Concentration (mg total product/L)	Mean Cell Density ($\times 10^4$ cells/mL) ^a			Percent Difference ^b
	24 Hr	48 Hr	72 Hr	
Control	4.6	22	118	---
0.65	4.5	23	124	+5
1.3	3.8*	22	116	-2
2.5	1.7*	9.1	54*	-54
5.0	0.81*	1.7*	3.2*	-97
10	0.56*	0.67*	1.0*	-99

^a Values are means of triplicate test chambers.

^b Percent difference as compared to the control was determined at 72 hours using the following equation:

$$\% \text{ difference} = (((\text{cell density of treatment}) - (\text{cell density of control})) / \text{cell density of control}) \times 100$$

* Significant reduction as compared to the control (Dunnett's test, $p < 0.05$).

NOTE: The target cell density at test initiation was 1.0×10^4 cells/mL.

Table 3. Replicate Cell Density for the Green Alga, *Pseudokirchneriella subcapitata*, During a 72-Hour Exposure to Allyl Alcohol 20906MB

Nominal Concentration (mg total product/L)	Rep	Cell Density ($\times 10^4$ cells/mL)		
		24 Hr	48 Hr	72 Hr
Control	A	4.8	20	120
	B	4.3	22	112
	C	4.7	23	121
0.65	A	4.7	22	130
	B	4.1	23	119
	C	4.8	24	122
1.3	A	4.1	22	124
	B	4.0	23	111
	C	3.3	22	112
2.5	A	1.8	8.9	53
	B	1.9	9.1	52
	C	1.4	9.4	58
5.0	A	0.56	1.9	2.6
	B	1.2	2.0	3.9
	C	0.67	1.3	3.1
10	A	0.67	0.67	0.78
	B	0.56	0.56	1.1
	C	0.44	0.78	1.2

Note: All cells were normal in appearance.

Table 4. Mean Area Under the Growth Curve Values for the Green Alga, *Pseudokirchneriella subcapitata*, Exposed to Allyl Alcohol 20906MB

Nominal Concentration (mg total product/L)	Mean Area Under Growth Curve ^a			Percent Difference ^b
	0-24 Hr	0-48 Hr	0-72 Hr	
Control	43	330	2,000	---
0.65	42	350	2,100	+5
1.3	34*	320	2,000	0
2.5	8.4*	110*	850*	-58
5.0	-2.3*	4.2*	39*	-98
10	-5.3*	-15*	-18*	-100

^a Values are means of triplicate test chambers and rounded to two significant figures.

^b Percent difference as compared to the control was determined at 72 hours using the following equation:

$$\% \text{ difference} = (((\text{area under growth curve of treatment}) - (\text{area under growth curve of control})) / \text{area under growth curve of control}) \times 100$$

* Significant reduction as compared to the control (Dunnett's test, $p < 0.05$).

Table 5. Mean Growth Rate Values for the Green Alga, *Pseudokirchneriella subcapitata*, Exposed to Allyl Alcohol 20906MB

Nominal Concentration (mg total product/L)	Mean Growth Rate (cells/mL/hour) ^a			Percent Difference ^b
	0-24 Hr	0-48 Hr	0-72 Hr	
Control	0.064	0.064	0.066	--
0.65	0.063	0.065	0.067	+2
1.3	0.055	0.065	0.066	0
2.5	0.022*	0.046*	0.055*	-17
5.0	-0.011*	0.011*	0.016*	-76
10	-0.025*	-0.0085*	0.00014*	-100

^a Values are means of triplicate test chambers and rounded to two significant figures.

^b Percent difference as compared to the control was determined at 72 hours using the following equation:

$$\% \text{ difference} = (((\text{growth rate of treatment}) - (\text{growth rate of control})) / \text{growth rate of control}) \times 100$$

* Significant reduction as compared to the control (Dunnett's test, $p < 0.05$).

Table 6. Calculated EC Values Based on Nominal Concentrations of Allyl Alcohol 20906MB During an Exposure of the Green Alga, *Pseudokirchneriella subcapitata*

Hour	EC Type	EC Value (mg total product/L)	95% Confidence Limits (mg total product/L)	NOEC ^a (mg total product/L)
24	E _b C ₅₀	2.2	2.0 and 2.3	0.65
	E _r C ₅₀	2.3	2.0 and 2.7	1.3
48	E _b C ₅₀	2.2	2.1 and 2.4	1.3
	E _r C ₅₀	3.3	2.7 and 3.8	1.3
72	E _b C ₅₀	2.4	2.3 and 2.4	1.3
	E _r C ₅₀	3.8	3.5 and 4.0	1.3

^a Highest test substance treatment showing no significant ($p = 0.05$) reduction as compared to the control.

Table 7. Calculated EC Values Based on the Adjusted Geometric Mean of the Measured Concentrations of Allyl Alcohol 20906MB During an Exposure of the Green Alga, *Pseudokirchneriella subcapitata*

Hour	EC Type	EC Value (mg/L)	95% Confidence Limits (mg/L)	NOEC ^a (mg/L)
24	E _b C ₅₀	2.09	1.95 and 2.23	0.343
	E _r C ₅₀	2.26	1.90 and 2.61	0.930
48	E _b C ₅₀	2.11	1.77 and 2.46	0.930
	E _r C ₅₀	5.14	4.79 and 5.50	0.930
72	E _b C ₅₀	2.25	2.21 and 2.30	0.930
	E _r C ₅₀	5.38	5.28 and 5.47	0.930

^a Highest test substance treatment showing no significant ($p = 0.05$) reduction as compared to the control.

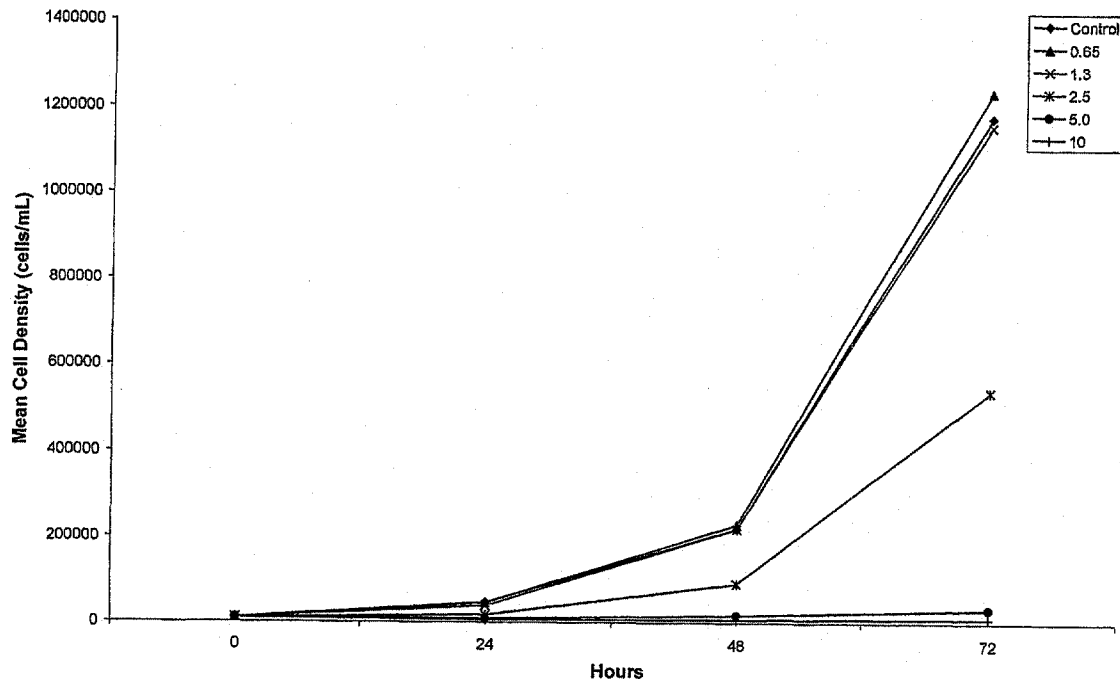
Table 8. Temperature and pH Measurements During the Exposure of *Pseudokirchneriella subcapitata* to Allyl Alcohol 20906MB

Nominal Concentration (mg total product/L)	0 Hr ^a		72 Hr ^b	
	Temp (°C)	pH	Temp (°C)	pH
Control	22.8	7.5	23.5	8.9
0.65	22.5	7.5	24.0	9.2
1.3	22.6	7.5	23.9	9.0
2.5	22.7	7.5	23.6	8.2
5.0	23.2	7.5	23.7	7.8
10	22.7	7.5	23.8	7.7

^a Measured in parent solutions.

^b Measured in replicate A.

Figure 1. Growth Curves for the Green Alga, *Pseudokirchneriella subcapitata*, During a 72-Hour Exposure to Allyl Alcohol 20906MB



ABC Study No. 48910

APPENDIX A. ABC REAGENT WATER CHARACTERIZATION

Chemical Characteristics of ABC Reagent Water Used by
ABC Laboratories' Chemical Development Group

August 2003 ABC Reagent Water Screen					
Chlorinated Hydrocarbons (µg/L)	2003	Historical Range 98-02	Elements (mg/L)	2003	Historical Range 98-02
DDE	<0.040	<0.040	Arsenic	<0.050	<0.010
DDD	<0.040	<0.040	Boron	<0.020	<0.020
DDT	<0.040	<0.040	Cadmium	<0.0050	<0.0050
Dieldrin	<0.040	<0.040	Chromium	<0.010	<0.010
α-BHC	<0.040	<0.040	Copper	<0.010	<0.010
β-BHC	<0.040	<0.040	Iron	<0.10	<0.10
γ-BHC	<0.040	<0.040	Lead	<0.0050	<0.0065
Δ-BHC	<0.040	<0.040	Mercury	<0.00050	<0.00060
Heptachlor epoxide	<0.040	<0.040	Nickel	<0.020	<0.020
Endrin	<0.040	<0.040	Selenium	<0.050	<0.0050
Methoxychlor	<0.040	<0.095	Silver	<0.0050	<0.0050
Toxaphene	<0.10	<3.8	Zinc	<0.020	<0.0020
Chlordane	<0.10	<0.47	Calcium	<1.0	
Endosulfan I	<0.040	<0.040	Magnesium	<1.0	
Endosulfan II	<0.040	<0.040	Potassium	<1.0	
Endosulfan sulfate	<0.040	<0.040	Sodium	<1.0	
Aroclor 1016	<0.10	<0.16			
Aroclor 1221	<0.10	<0.16	<u>Organophosphate (µg/L)</u>		
Aroclor 1248	<0.10	<0.16	Diazinon	<1.0	<1.0
Aroclor 1232	<0.10	<0.16	Parathion	<1.0	<1.0
Aroclor 1242	<0.10	<0.16	Malathion	<1.0	<1.0
Aroclor 1254	<0.10	<0.16	Ethion	<1.0	<1.0
Aroclor 1260	<0.10	<0.16	Disulfoton	<1.0	<1.0
Aldrin	<0.040	<0.032	Azinphos ethyl	<1.0	<1.0
Endrin aldehyde	<0.040	<0.032	Demeton, Total	<1.0	<1.0
Endrin Ketone	<0.040	<0.032			
Heptachlor	<0.040	<0.032	<u>Miscellaneous (mg/L)</u>		
2,4,5-TP (silvex)	<50	<2.0	Nitrite N	<0.030	<0.050
2,4-D	<250	<10	Nitrate N	<0.030	<0.050-0.17
			Total Phosphorus as P	<0.050	<0.010-0.60

Note: Data supporting these values are on file at ABC Laboratories. Less than (<) values indicate recovery was below the limit of detection.

APPENDIX B. PROTOCOL, AMENDMENTS, AND DEVIATION

ABC Study No. 48910

**Toxicity of Allyl Alcohol 20906MB (Lyondell lot
number CX30609214) to the Unicellular Green Alga,
*Pseudokirchneriella subcapitata***

ABC Study No. 48910

This protocol complies with
OECD Guideline 201

This protocol is based upon ABC generic protocol E101.

1.0 STUDY TITLE

Toxicity of Allyl Alcohol 20906MB (Lyondell lot number CX30609214) to the Unicellular Green Alga, *Pseudokirchneriella subcapitata*

2.0 OBJECTIVE

The primary objective of this test is to determine the 72-hour EC_{50} (E_bC_{50} and/or E_rC_{50}) of the test substance to the unicellular green alga, *Pseudokirchneriella subcapitata*, under static test conditions. An EC_{50} (E_bC_{50} and/or E_rC_{50}) is the approximate concentration of the test substance that inhibits 50% of algal growth (measured as biomass or growth rate), relative to the control. In addition, the no-observed-effect concentration (NOEC) at 72 hours will be determined, if possible.

3.0 STUDY SPONSOR

Lyondell Chemical Company
One Houston Center, Suite 1600
1221 McKinney
Houston, TX 77010

Sponsor Representative: David F. Ludwig, Ph.D.
BBL Sciences
326 First Street, Suite 200
Annapolis, MD 21403-2678
TEL: (410) 295-1205 FAX: (410) 295-1225

4.0 TESTING FACILITY AND STUDY DIRECTOR ADDRESS

ABC Laboratories, Inc.
7200 E. ABC Lane
Columbia, Missouri 65202

Study Director: Stephen L. Hicks
TEL: (573) 443-9053 FAX: (573) 443-9089
EMAIL: hickss@abclabs.com

5.0 PROPOSED SCHEDULE

PROPOSED EXPERIMENTAL START DATE: June 2004
PROPOSED EXPERIMENTAL COMPLETION DATE: June 2004

6.0 TEST PROTOCOL

The test protocol that follows is based on method 201 of the OECD Guidelines for Testing of Chemicals (1).

7.0 TEST AND REFERENCE SUBSTANCES

7.1 Test Substance

The test substance will be Allyl Alcohol 20906MB (Lyondell lot number CX30609214). The following sample information and chemical/physical properties should be provided with the test substance sample or before its shipment: batch/lot number, sample expiration date, physical description, purity (including certificate of analysis), stability, suggested storage conditions, water and organic solvent solubility, vapor pressure, available toxicity information, a Material Safety Data Sheet (MSDS) or its equivalent, and handling precautions.

7.2 Reference Substance

The test substance will be used as the reference substance (i.e., analytical standard).

7.3 Sample Characterization and Retention

Characterization, stability, and solubility studies will be the responsibility of the Sponsor unless otherwise contracted to ABC Laboratories, Inc. The test substance will be properly disposed of following completion of its use at ABC Laboratories, Inc., unless arrangements for retention or return are made by the Sponsor. Archival of a retention sample will also be the Sponsor's responsibility.

7.4 Test Substance Preparation/Addition

The test concentrations will be prepared on a weight/volume basis unless otherwise specified. Test concentrations will be based on the active ingredient (ai) percentage, unless otherwise directed by the Sponsor. If toxicity is not observed at or below the maximum solubility of the test substance in the test medium, the highest test concentration definitively tested will be the maximum solubility of the test substance in the test medium or 1000 milligrams per liter (mg ai/L), whichever is lower.

8.0 TEST SYSTEM

8.1 Species

The test species will be the unicellular green alga, *Pseudokirchneriella subcapitata*, formerly known as *Selenastrum capricornutum*. The species will be initially identified by the supplier and then periodically inspected to insure the purity and identity of the culture.

8.2 Justification

When freshwater algal toxicity data are generated following OECD guideline 201, *Pseudokirchneriella subcapitata* is one of the recommended species.

8.3 Source

Pseudokirchneriella subcapitata (UTEX 1648) will be obtained from an established ABC Laboratories' culture which originated with an inoculum received from the University of Texas, Austin, Texas.

8.4 Age

Transfers will be made regularly into fresh algal nutrient medium to provide 5- to 10-day old cultures for test inoculations.

8.5 Culture

The algae will be cultured in freshwater algal nutrient medium under continuous illumination of approximately 8600 lux at a temperature of $24 \pm 2^\circ\text{C}$.

9.0 TEST MEDIUM

The test medium will be filtered (0.45 micrometers) freshwater algal growth medium prepared with ABC reagent water and reagent grade chemicals (2). The pH of the medium prior to inoculation will be 7.5 ± 0.1 and may be adjusted to this pH with 0.1N NaOH or HCl prior to test substance addition. Depending upon the situation, the pH may be adjusted following test substance addition, but prior to inoculation of algae. ABC reagent water used in the preparation of the algal growth medium is chemically characterized annually per ABC SOP to verify that it is free of contaminants that might interfere with test results.

10.0 TEST PROCEDURES

Generally two toxicity tests will be conducted, a range-finding and definitive test. The range-finding test is an abbreviated toxicity test employing widely spaced test concentrations to define the approximate range within which the test substance produces a gradient from nontoxic to toxic effects. The range-finding test is conducted using the same basic procedures and conditions as those used during definitive tests. Results of the range-finding test(s) guide selection of the test concentrations for the definitive test, the purpose of which is provide a precise estimate of the 72-hour median effective concentration (EC_{50}) of the test substance which affects the growth of this alga.

10.1 Range-Finding Test

The range-finding test(s) will be initiated by inoculating at least one flask per test substance concentration with a predetermined aliquot of algal inoculum. The test concentrations will typically cover several orders of magnitude (e.g., 1.0, 10, 100, 1000 mg/L). Typically, test condition parameters such as light intensity, oscillation rate, test solution pH and temperature will be measured at test initiation and termination. Additional or fewer measurements may be made at the discretion of the Study Director. At a minimum, cell counts will be determined after approximately 72 hours of exposure.

10.2 Definitive Test

10.2.1 Experimental Design

The definitive test will consist of one or more control treatments and a geometric series (ratio between concentrations ≤ 2.2) of at least five test substance concentrations. If any vehicle other than test medium or water is present in any of the test vessels, a vehicle control will be maintained concurrently. The vehicle control will possess the greatest concentration of vehicle present in any of the treatments. If a vehicle is utilized, the concentration of vehicle will not exceed 100 μ L/L. Definitive test concentrations and vehicle control, if needed, will be specified by protocol amendment. All test chambers will be labeled with the following information for identification purposes: ABC study number, treatment (e.g., control, vehicle control, level 1, level 2, etc.), replicate (e.g., A, B, etc.) and grid position.

The definitive test will generally be conducted in 250-mL Erlenmeyer flasks fitted with foam stoppers to permit gas exchange and to prevent contamination. If 250-mL flasks are used, each flask will contain 100 mL of test solution. The size of the Erlenmeyer flasks is not critical, but the sample-to-volume ratio should not exceed 50%. Flasks used in testing will be cleaned and sterilized (autoclaved) according to the ABC Laboratories' SOP. Three replicates will be used for each control, vehicle control (if necessary), and test substance concentration. Each replicate will be inoculated with algae and placed on a rotary shaker at approximately 100 revolutions per minute. For the control of bias among replicates, test flasks used during the definitive test will be assigned to the testing area using a computer-generated randomization table. The algal inoculum will be from a 5 to 10-day-old stock culture or a culture with a sufficient cell density to yield a final inoculum density of 1×10^6 cells/mL. The test algae will be inoculated into the test flasks within 30 minutes after preparation of test solutions to yield an approximate initial cell density of 1×10^4 cells/mL. The test flasks will then be incubated in a temperature-controlled enclosure illuminated continuously for 72 hours. This route of administration was selected to comply with OECD guideline 201.

A limit test may be performed at a concentration of 1000 mg/L or at a concentration equal to the solubility of the substance in the medium for situations in which the algal growth inhibition is estimated to be less than 50% at this maximum test concentration. The limit test will be performed in triplicate, with the same number of controls. If, in a limit test, a mean decrease of 25% or more is found in either biomass or growth rate between the limit test and the control, a full test should be carried out.

10.2.2 Lighting and Oscillation

Throughout the test, cool-white fluorescent bulbs that provide 8600 ± 860 lux and continuously swirled on an orbital shaker table at approximately 100 rpm will illuminate the test flasks continuously. Daily light readings (measured at the level of the test solutions) and shaker oscillation rate will be measured and recorded.

10.2.3 Chemical/Physical Parameters

Temperature of the environmental chamber will be measured continuously during the definitive test. Temperature and pH will be measured at 0 and

72 hours in the control, vehicle control (if necessary) and all test substance concentrations. Measurement at test initiation (0-hour) will be conducted on all parent solutions (prior to distribution of the solutions to the test vessels). Measurements at test termination will be conducted on at least one replicate of the control, vehicle control (if necessary), and all test substance concentrations. The temperature should be $24 \pm 2^\circ\text{C}$ and the pH of the solutions should not normally deviate more than 1 pH unit during the test.

10.2.4 Biological Data

Cell density will be determined for each replicate of the control, vehicle control (if necessary), and each test concentration at 24, 48, and 72 hours (± 1 hour from test initiation) to evaluate algal growth (inhibition or enhancement). Cell density may also be determined at 0 hour for each replicate of the control and vehicle control (if necessary) to confirm initial cell densities. Cell density determinations will be accomplished using a hemacytometer and an optical microscope. In addition to cell density determinations, microscopic examination will be conducted to determine if there are any morphological or physical effects on the algal cells. Unusual cell shapes, color differences, flocculation, adherence of algae to test chambers, or aggregation of algal cells will be noted.

10.2.5 Analytical Confirmation

The concentrations of the test substance will be measured in all control and test substance treatments at 0 and 72 hours. Time 0-hour samples will be collected from parent solutions. Time 72-hour samples will be collected after combining replicate solutions by treatment. A minimum of two fortification spikes (quality control samples) will be prepared and analyzed with each sample set. An extra replicate containing the lowest test concentration without algae may be included and analyzed at test termination to determine if the test substance may have been incorporated into the algal biomass during the period of the test. Additional flasks per treatment may be prepared if necessary for analytical confirmation purposes.

The Sponsor will submit an appropriate analytical method to conduct the analysis and the method will be validated before the definitive test is started. The analytical method will be described by protocol amendment to this protocol after validation.

11.0 ANALYSIS OF RESULTS

The results of the definitive test will be examined to determine those concentrations that inhibit or enhance growth of the test algae. Generally, results will be reported using the overall mean measured concentrations (mean of 0- and 72-hour measured concentrations) when test solutions have been analyzed, unless otherwise requested by the study sponsor. When test solutions have not been analyzed, results will be reported using the nominal concentrations.

The results of the definitive test will be statistically analyzed for 24-, 48-, and 72-hour EC_{50} (E_bC_{50} and/or E_rC_{50}) values and corresponding 95% confidence limits, if data permit. These values will be determined by the SAS nonlinear modeling procedure (four parameter logistic model with two parameters fixed). The method used will be identified in the report. In addition, the no-observed-effect concentration (NOEC) for at least 72 hours will be determined, if possible, by a one-way analysis of variance (ANOVA) and a multiple means comparison test using the individual replicates values of the areas under the growth curves or the specific growth rates. Additional effect concentrations can be estimated, if desired.

12.0 TEST ACCEPTABILITY CRITERIA

The number of algal cells in the control(s) at test termination should be at least 16 times the number initially inoculated to verify logarithmic phase growth. Unless the maximum test concentration of test substance is tested (i.e., at maximum solubility or 1,000 mg/L), one test concentration should exhibit $\leq 50\%$ decrease in growth and one concentration should exhibit $\geq 50\%$ decrease in growth relative to the control(s).

13.0 REPORT

A final report will be submitted to the Sponsor and will include, but not be limited to, the following:

- Study dates, name, and address of test facility.
- Objectives and test procedures as stated in approved protocol.
- A description of the experimental design along with a description of and reference to any statistical methods used for data analysis.
- Description of test substance (e.g., date of receipt, storage conditions, method of preparing stock and/or test solution and, if available, purity, physical characteristics,

water and organic solvent solubility) and identification of the reference substance, if applicable.

- Description of test conditions during the study (e.g., vehicle used, dilution water, test temperature, lighting, and pH).
- Description of methods used during the study.
- Description of test system (e.g., source, culture techniques, etc.).
- Summary of the data and a statement of the conclusions drawn from any data analyses, if appropriate.
- Description of any protocol deviations.
- Location of raw data.
- List of all study personnel.
- GLP compliance statement by the Study Director and a statement by ABC Laboratories' Quality Assurance Unit.

14.0 PROTOCOL AMENDMENTS AND DEVIATIONS

The Study Director, upon approval of the Sponsor Representative, may make amendments to this protocol. All amendments will describe the change(s), the reason(s) for the amendment, and the effect on the study, if any. All amendments will be signed and dated by at least the Study Director and maintained with the protocol.

In the event of a protocol deviation, a written description of the deviation including the reason for the deviation and any impact on the study as a result of the deviation will be submitted to the Sponsor Representative. All deviations will be signed and dated by at least the Study Director and maintained with the protocol.

15.0 QUALITY ASSURANCE

ABC's Quality Assurance Unit will inspect one or more critical phases to assure that equipment, personnel, procedures, and records conform to the guidelines listed in this protocol. The results of these inspections will be reported to the Study Director and ABC management. The draft and final reports will be reviewed for protocol and GLP compliance, as well as to assure that the methods and standard operating procedures used were followed.

A signed statement will be included in the report specifying types of inspections made, the dates inspections were made, and the dates inspections were reported to the Study Director and management.

16.0 GLP COMPLIANCE

This study will be conducted in accordance with OECD Principles of Good Laboratory Practice (3). The report will contain a statement attesting to that fact.

17.0 RECORDS

Records to be maintained will include, but not be limited to, test substance receipt; solution preparations and dilutions; instrument logbooks detailing calibration and maintenance; facility records (kept at ABC); material control identification numbers for all instruments used; storage of test substance, solutions, and samples; and weights and volumes. All original raw data collected during this study will be maintained at ABC Laboratories until finalization of the study. Upon completion of the study, all original raw data will be submitted to the Sponsor along with the final report. A copy of the final report, copies of all raw data from the study, and all original facility records will be kept on file in ABC Laboratories' archives.

18.0 SPECIMEN DISPOSAL

Following finalization of the report, disposition of all specimens (i.e., any material derived from the test system for examination, analysis, or retention) generated during the conduct of the test will be completed in a timely manner. Retention specimens holding time will be based on stability information provided by the Sponsor or by stability data generated by ABC Laboratories. Retention specimens will be returned to the Sponsor unless archiving is contracted with ABC laboratories. Documentation of specimen disposal will be retained with study records in ABC Laboratories' Archive.

19.0 REFERENCES

- (1) Organization for Economic Cooperation and Development (OECD). June 7, 1984. OECD Guidelines for Testing of Chemicals. Algae, Growth Inhibition Test, OECD Guideline No. 201.
- (2) American Society for Testing and Materials (ASTM). 1997. Standard Guide for Conducting Static 96-h Toxicity Tests with Microalgae. ASTM Designation E1218-97a, 14 pp.
- (3) Organization for Economic Cooperation and Development. 1997. Decision of the Council, Revised Principles of GLP [C(97)186/Final].

ABC STUDY No. 48910

Page ¹⁰ 12 of 11

PROTOCOL APPROVAL

ABC Laboratories' Study Director

Name (signed): Stephen L. Hicks Date: 08 JUN 04

Name/Title: Stephen L. Hicks / Research Scientist

Sponsor Representative

Name (signed): David Ludwig Date: 7 JUN 04

Name/Title: David Ludwig, Ph.D.

ABC Laboratories' OAU Protocol Review for GLP Compliance

Name (signed): Jessica Dutton Date: 09 JUN 04

Name/Title: Jessica Dutton / Quality Assurance Officer II

Test Facility Management

Name (signed): Jon E. Rhodes For Date: 08 JUN 04

Name/Title: Jon E. Rhodes / Biological Services Director

① Page number correction. SH 08 JUN 04

PROTOCOL AMENDMENT

PROTOCOL TITLE: Toxicity of Allyl Alcohol 20906MB (Lyondell lot number XC30609214) to the Unicellular Green Alga, *Pseudokirchneriella subcapitata*

LABORATORY: ABC Laboratories, Inc.

SPONSOR: Lyondell Chemical Company

ABC STUDY NO.: 48910

AMENDMENT NO.: 1

EFFECTIVE DATE: 20 OCT 04

1. Protocol Section: 3.0 Study Sponsor

Sponsor Representative is changed to the following:

Jim Bootman
Bootman Chemical Safety Ltd
Diss Business Centre
Diss, Norfolk
IP21 4HD, U.K.
Tel: (+44) - (0)1379 640534 Email: jim@bootmanchem.com

Reason: Requested by Study Sponsor.

Effect on Study: None

2. Protocol Section: 4.0 Testing Facility and Study Director Address

Environmental Chemistry, Inc. (ECI) is added as a testing facility. ECI will perform the analytical confirmation. The address for ECI is as follows:

Environmental Chemistry, Inc.
2525 West Bellfort, Suite 175
Houston, Texas 77054
Contact: Carolyn Elmore Tel: 713-666-0020

Reason: Requested by Study Sponsor.

Effect on Study: None

ABC Study No. 48910
Amendment No. 1
Page 2 of 2

3. Protocol Section: 10.2.1 Experimental Design

The definitive test will be conducted with the following nominal concentrations: 0 (control), 0.65, 1.3, 2.5, 5.0, and 10 mg total product/L. The control and test substance treatment solutions will be freshly prepared at 24 hours and all surviving daphnids will be transferred by pipet from old solutions to new solutions.

Reason: Identify the nominal concentrations selected for the definitive test and provide detail regarding the renewal of test solutions.

Effect on Study: None

4. Protocol Section: 10.2.5 Analytical Confirmation

The following treatments will be sampled during the exposure:

- 1) 0 hour: control, 0.65, 1.3, 2.5, 5.0, and 10 mg total product/L
- 2) 72 hour: control, 0.65 (biotic and abiotic), 1.3, 2.5, 5.0, and 10 mg total product/L

The 0 hour solution samples will be collected from the parent solutions. The 72 hour solutions will be sampled after combining replicate test solutions by treatment. Samples will be acidified to pH <2.0 with hydrochloric acid, placed into 40-mL glass vials so that no headspace remains, and sealed. All samples will be refrigerated prior to shipment for analysis. Samples will be sent chilled, with artificial ice, to ECI for analysis.

Reason: Identify treatments sampled and sampling procedure.

Effect on Study: None

ABC LABORATORIES' STUDY
DIRECTOR'S SIGNATURE:

Stephen J. Hicks

DATE: 20 OCT 04

STUDY SPONSOR'S
SIGNATURE:

J. B. E.

DATE: 8/11/04

PROTOCOL AMENDMENT

PROTOCOL TITLE: Toxicity of Allyl Alcohol 20906MB (Lyondell lot number XC30609214) to the Unicellular Green Alga, *Pseudokirchneriella subcapitata*

LABORATORY: ABC Laboratories, Inc.

SPONSOR: Lyondell Chemical Company

ABC STUDY NO.: 48910

AMENDMENT NO.: 2

EFFECTIVE DATE: 26 OCT 04

I. Protocol Section: Amendment No. 1; Item No. 3

The following sentence is deleted: "The control and test substance treatment solutions will be freshly prepared at 24 hours and all surviving daphnids will be transferred by pipet from old solutions to new solutions."

The "Reason" for the amendment should read as follows: Identify the nominal concentrations selected for the definitive test.

Reason: Correction.

Effect on Study: None

ABC LABORATORIES' STUDY

DIRECTOR'S SIGNATURE:

Stephen A. Hicks

DATE: 02 NOV 04

STUDY SPONSOR'S

SIGNATURE:

J. E. [Signature]

DATE: 8/11/04

PROTOCOL AMENDMENT

PROTOCOL TITLE: Toxicity of Allyl Alcohol 20906MB (Lyondell lot number CX30609214) to the Unicellular Green Alga, *Pseudokirchneriella subcapitata*

LABORATORY: ABC Laboratories, Inc.

SPONSOR: Lyondell Chemical Company

ABC STUDY NO.: 48910

AMENDMENT NO.: 3

EFFECTIVE DATE: 25 JAN 05

1 Protocol Section: Amendment No. 1; Protocol Title

The Lyondell lot number is changed to CX30609214.

Reason: Correction.

Effect on Study: None

2. Protocol Section: Amendment No. 2; Protocol Title

The Lyondell lot number is changed to CX30609214.

Reason: Correction.

Effect on Study: None

ABC LABORATORIES' STUDY

DIRECTOR'S SIGNATURE:

Stephen J. Hicks

DATE: 10 Feb 05

**STUDY SPONSOR'S
SIGNATURE:**

J. B. [unclear]

DATE: 26/1/05

PROTOCOL DEVIATION

PROTOCOL TITLE: Toxicity of Allyl Alcohol 20906MB (Lyondell lot number CX30609214) to the Unicellular Green Alga, *Pseudokirchneriella subcapitata*

LABORATORY: ABC Laboratories, Inc.

SPONSOR: Lyondell Chemical Company

ABC STUDY NO.: 48910

DEVIATION NO.: 1

EFFECTIVE DATE: 16 FEB 05

1. Protocol Section: 10.2.4 Biological Data

Test initiation was at 3:30 pm on October 26, 2004, and the cell density measurements at 72 hours were performed at 2:10 pm which was outside the observation time range of ± 1 hour from test initiation.

Reason: Cell density measurements were inadvertently performed 20 minutes prior to the observation point required by the protocol.

Effect on Study: The deviation has no effect on the integrity of the study.

**ABC LABORATORIES' STUDY
DIRECTOR'S SIGNATURE:**

Stephen L Hicks

DATE: 16 Feb 05

APPENDIX C. ANALYTICAL DATA

ABC Study No. 48910

Environmental Chemistry, Inc.
2525 West Bellfort, Suite 175
Houston, Texas 77054

Phone 713-666-0020
Fax 713-666-4176

ANALYTICAL REPORT

Equistar - Lyondell Chemical Company -Corporate
One Houston Center, Suite 1600
1221 McKinney Street
Houston, Texas 77010
Attention: Marcy Banton
Project: Allyl Alcohol Project

Job: 042637
Received: 11/10/04
Reported: 12/06/04
P. O. 4400544831
48909

042637- 1 0-Hr Control
Collected: 10/26/04 by Steve Hicks

Analyte	Result	Dilution	S.Q.L.	Method	Code
<i>Miscellaneous Volatiles by GC/MS</i>					
Allyl alcohol ug/L	ND	1	40	8260B	VOAM
Dibromofluoromethane, Surrogate Recovery, %	99				
Toluene-d8, Surrogate Recovery, %	96				
4-Bromofluorobenzene, Surrogate Recovery, %	101				

The holding time expired for Volatile Organics analysis before the sample was received at the laboratory. The client was notified and instructed the lab to proceed with the analysis.

042637- 2 0-Hr Level 1 (0.65 mg/L)
Collected: 10/26/04 by Steve Hicks

Analyte	Result	Dilution	S.Q.L.	Method	Code
<i>Miscellaneous Volatiles by GC/MS</i>					
Allyl alcohol ug/L	424	1	40	8260B	VOAM
Dibromofluoromethane, Surrogate Recovery, %	99				
Toluene-d8, Surrogate Recovery, %	97				
4-Bromofluorobenzene, Surrogate Recovery, %	101				

The holding time expired for Volatile Organics analysis before the sample was received at the laboratory. The client was notified and instructed the lab to proceed with the analysis.

042637- 3 0-Hr Level 2 (1.3 mg/L)
Collected: 10/26/04 by Steve Hicks

Analyte	Result	Dilution	S.Q.L.	Method	Code
<i>Miscellaneous Volatiles by GC/MS</i>					
Allyl alcohol ug/L	1,320	1	40	8260B	VOAM
Dibromofluoromethane, Surrogate Recovery, %	97				
Toluene-d8, Surrogate Recovery, %	96				
4-Bromofluorobenzene, Surrogate Recovery, %	100				

The holding time expired for Volatile Organics analysis before the sample was received at the laboratory. The client was notified and instructed the lab to proceed with the analysis.

042637- 4 0-Hr Level 3 (2.5 mg/L)
Collected: 10/26/04 by Steve Hicks

Analyte	Result	Dilution	S.Q.L.	Method	Code
<i>Miscellaneous Volatiles by GC/MS</i>					
Allyl alcohol ug/L	3,750	5	200	8260B	VOAM

Page AR-1 Continued on Page AR-2 Unless noted, results are reported on an as-received basis.
Results below the Sample Quantitation Limit (SQL) are reported as ND.

Environmental Chemistry, Inc.
2525 West Bellfort, Suite 175
Houston, Texas 77054

Phone 713-666-0020
Fax 713-666-4176

ANALYTICAL REPORT

Equistar - Lyondell Chemical Company -Corporate
Project: Allyl Alcohol Project

Job: 042637

042637- 4 0-Hr Level 3 (2.5 mg/L)
Collected: 10/26/04 by Steve Hicks

Analyte	Result	Dilution	S.Q.L.	Method	Code
<i>Miscellaneous Volatiles by GC/MS</i>					
Dibromofluoromethane, Surrogate Recovery, %	120			8280B	VOAM
Toluene-d8, Surrogate Recovery, %	101				
4-Bromofluorobenzene, Surrogate Recovery, %	94				

The holding time expired for Volatile Organics analysis before the sample was received at the laboratory. The client was notified and instructed the lab to proceed with the analysis.

042637- 5 0-Hr Level 4 (5.0 mg/L)
Collected: 10/26/04 by Steve Hicks

Analyte	Result	Dilution	S.Q.L.	Method	Code
<i>Miscellaneous Volatiles by GC/MS</i>					
Allyl alcohol ug/L	9,000	5	200	8280B	VOAM
Dibromofluoromethane, Surrogate Recovery, %	107				
Toluene-d8, Surrogate Recovery, %	97				
4-Bromofluorobenzene, Surrogate Recovery, %	101				

The holding time expired for Volatile Organics analysis before the sample was received at the laboratory. The client was notified and instructed the lab to proceed with the analysis.

042637- 6 0-Hr Level 5 (10 mg/L)
Collected: 10/26/04 by Steve Hicks

Analyte	Result	Dilution	S.Q.L.	Method	Code
<i>Miscellaneous Volatiles by GC/MS</i>					
Allyl alcohol ug/L	12,800	20	800	8280B	VOAM
Dibromofluoromethane, Surrogate Recovery, %	117				
Toluene-d8, Surrogate Recovery, %	98				
4-Bromofluorobenzene, Surrogate Recovery, %	96				

The holding time expired for Volatile Organics analysis before the sample was received at the laboratory. The client was notified and instructed the lab to proceed with the analysis.

042637- 7 72-Hr Control Solution
Collected: 10/29/04 by Steve Hicks

Analyte	Result	Dilution	S.Q.L.	Method	Code
<i>Miscellaneous Volatiles by GC/MS</i>					
Allyl alcohol ug/L	ND	1	40	8280B	VOAM
Dibromofluoromethane, Surrogate Recovery, %	98				
Toluene-d8, Surrogate Recovery, %	98				
4-Bromofluorobenzene, Surrogate Recovery, %	98				

Page AR-2 Continued on Page AR-3 Unless noted, results are reported on an as-received basis.
Results below the Sample Quantitation Limit (SQL) are reported as ND.

ABC Study No. 48910

Environmental Chemistry, Inc.
2525 West Belfort, Suite 175
Houston, Texas 77054

Phone 713-666-0020
Fax 713-666-4176

ANALYTICAL REPORT

Equistar - Lyondell Chemical Company -Corporate
Project: Allyl Alcohol Project

Job: 042637

042637- 8 72-Hr Level 1 Biotic Solution (0.65 mg/L)
Collected: 10/29/04 by Steve Hicks

Analyte	Result	Dilution	S.Q.L.	Method	Code
Miscellaneous Volatiles by GC/MS				8260B	VOAM
Allyl alcohol ug/L	341	1	40		
Dibromofluoromethane, Surrogate Recovery, %	96				
Toluene-d8, Surrogate Recovery, %	99				
4-Bromofluorobenzene, Surrogate Recovery, %	103				

042637- 9 72-Hr Level 1 ABIOTIC Solution (0.65 mg/L)
Collected: 10/29/04 by Steve Hicks

Analyte	Result	Dilution	S.Q.L.	Method	Code
Miscellaneous Volatiles by GC/MS				8260B	VOAM
Allyl alcohol ug/L	348	1	40		
Dibromofluoromethane, Surrogate Recovery, %	95				
Toluene-d8, Surrogate Recovery, %	95				
4-Bromofluorobenzene, Surrogate Recovery, %	101				

042637- 10 72-Hr Level 2 Solution (1.3 mg/L)
Collected: 10/29/04 by Steve Hicks

Analyte	Result	Dilution	S.Q.L.	Method	Code
Miscellaneous Volatiles by GC/MS				8260B	VOAM
Allyl alcohol ug/L	803	1	40		
Dibromofluoromethane, Surrogate Recovery, %	102				
Toluene-d8, Surrogate Recovery, %	96				
4-Bromofluorobenzene, Surrogate Recovery, %	100				

042637- 11 72-Hr Level 3 Solution (2.5 mg/L)
Collected: 10/29/04 by Steve Hicks

Analyte	Result	Dilution	S.Q.L.	Method	Code
Miscellaneous Volatiles by GC/MS				8260B	VOAM
Allyl alcohol ug/L	1,900	1	40		
Dibromofluoromethane, Surrogate Recovery, %	96				
Toluene-d8, Surrogate Recovery, %	99				
4-Bromofluorobenzene, Surrogate Recovery, %	97				

Page AR-3 Continued on Page AR-4 Unless noted, results are reported on an as-received basis.
Results below the Sample Quantitation Limit (SQL) are reported as ND.

Environmental Chemistry, Inc.
2525 West Bellfort, Suite 175
Houston, Texas 77054

Phone 713-666-0020
Fax 713-666-4176

ANALYTICAL REPORT

Equistar - Lyondell Chemical Company -Corporate
Project: Allyl Alcohol Project

Job: 042637

042637- 12 72-Hr Level 4 Solution (5.0 mg/L)
Collected: 10/29/04 by Steve Hicks

<i>Analyte</i>	<i>Result</i>	<i>Dilution</i>	<i>S.Q.L.</i>	<i>Method</i>	<i>Code</i>
<i>Miscellaneous Volatiles by GC/MS</i>					
Allyl alcohol ug/L	4,950	5	200	8280B	VOAM
Dibromofluoromethane, Surrogate Recovery, %	104				
Toluene-d8, Surrogate Recovery, %	102				
4-Bromofluorobenzene, Surrogate Recovery, %	103				

042637- 13 72-Hr Level 5 Solution (10 mg/L)
Collected: 10/29/04 by Steve Hicks

<i>Analyte</i>	<i>Result</i>	<i>Dilution</i>	<i>S.Q.L.</i>	<i>Method</i>	<i>Code</i>
<i>Miscellaneous Volatiles by GC/MS</i>					
Allyl alcohol ug/L	7,910	5	200	8280B	VOAM
Dibromofluoromethane, Surrogate Recovery, %	104				
Toluene-d8, Surrogate Recovery, %	96				
4-Bromofluorobenzene, Surrogate Recovery, %	101				

Environmental Chemistry, Inc. has a quality system in place that meets the standards of the National Environmental Laboratory Accreditation Program (NELAP) and received accreditation in 2001. Analyses for accredited analytes are performed in accordance with NELAP requirements unless noted by exception in this report. This report consists of the following sections: Analytical Report (AR), Report Summary (RS), and Quality Control (QC).

Page AR-4 End of AR Unless noted, results are reported on an as-received basis.
Results below the Sample Quantitation Limit (SCL) are reported as ND.

Environmental Chemistry, Inc.
2525 West Bellfort, Suite 175
Houston, Texas 77054

Phone 713-666-0020
Fax 713-666-41746

QUALITY CONTROL REPORT

Job: 042637

VOAM Miscellaneous Volatiles by GC/MS

Type	GC/MS	Method(s)	8260B SW-846;	824EPA600	Batch	20041113-LAM-2	ug/L	RPD	% Rec	Mean \pm 3 SD
Method Blank:					Result	SOL				
Allyl alcohol		Heated Purge			ND	40				
Benzene		Heated Purge			ND	1				
Chlorobenzene		Heated Purge			ND	5				
1,1-Dichloroethene		Heated Purge			ND	5				
Trichloroethene		Heated Purge			ND	5				
Toluene		Heated Purge			103					
Dibromofluoromethane, Surrogate Recovery, %		Heated Purge			97					
Toluene-d8, Surrogate Recovery, %		Heated Purge			102					
4-Bromofluorobenzene, Surrogate Recovery, %		Heated Purge								
Lab Control Sample Recovery:					Before	Added	Solvent			Limits
Allyl alcohol					ND	500	537		107.4	
Benzene					ND	50	52		104.0	
Chlorobenzene					ND	50	55		110.0	
1,1-Dichloroethene					ND	50	49		98.0	
Trichloroethene					ND	50	52		104.0	
Toluene					ND	50	51		122.0	
Dibromofluoromethane, Surrogate Recovery, %					ND	50	50		100.0	-
Toluene-d8, Surrogate Recovery, %					ND	50	50		100.0	-
4-Bromofluorobenzene, Surrogate Recovery, %					ND	50	50		100.0	-
Matrix Spike Duplicate:					MS	MSD				
Allyl alcohol	042637-8				1360	1120		19.4		
Benzene	042637-8				95	95		0.0		
Chlorobenzene	042637-8				98	102		0.1		
1,1-Dichloroethene	042637-8				91	89		2.2		
Trichloroethene	042637-8				105	102		2.9		
Toluene	042637-8				97	104		7.0		
Dibromofluoromethane, Surrogate Recovery, %	042637-8				48	47		2.1		
Toluene-d8, Surrogate Recovery, %	042637-8				52	51		1.9		
4-Bromofluorobenzene, Surrogate Recovery, %	042637-8				50	51		2.0		
Matrix Spike Recovery:					Before	Added	Solvent			
Allyl alcohol	042637-8				341	500	1350		203.8*	
Benzene	042637-8				ND	100	95		95.0	
Chlorobenzene	042637-8				ND	100	96		96.0	
1,1-Dichloroethene	042637-8				ND	100	91		91.0	
Trichloroethene	042637-8				ND	100	105		105.0	
Toluene	042637-8				ND	100	97		97.0	
Dibromofluoromethane, Surrogate Recovery, %	042637-8				ND	50	48		95.0	86.6-112.1
Toluene-d8, Surrogate Recovery, %	042637-8				ND	50	52		104.0	81.6-117.3
4-Bromofluorobenzene, Surrogate Recovery, %	042637-8				ND	50	50		100.0	87.3-115.6
Allyl alcohol	042637-8				341	500	1120		155.8*	
Benzene	042637-8				ND	100	95		95.0	
Chlorobenzene	042637-8				ND	100	102		102.0	
1,1-Dichloroethene	042637-8				ND	100	89		89.0	
Trichloroethene	042637-8				ND	100	102		102.0	
Toluene	042637-8				ND	100	104		104.0	
Dibromofluoromethane, Surrogate Recovery, %	042637-8				ND	50	47		94.0	86.6-112.1
Toluene-d8, Surrogate Recovery, %	042637-8				ND	50	51		102.0	81.6-117.3
4-Bromofluorobenzene, Surrogate Recovery, %	042637-8				ND	50	51		102.0	87.3-115.6

VOAM Miscellaneous Volatiles by GC/MS

Type	GC/MS	Method(s)	8260B SW-846;	824EPA600	Batch	20041118-LAM-1	ug/L	RPD	% Rec	Mean \pm 3 SD
Method Blank:					Result	SOL				
Allyl alcohol		Heated Purge			ND	40				
Benzene		Heated Purge			ND	1				
Chlorobenzene		Heated Purge			ND	5				
1,1-Dichloroethene		Heated Purge			ND	5				
Trichloroethene		Heated Purge			ND	5				
Toluene		Heated Purge			99					
Dibromofluoromethane, Surrogate Recovery, %		Heated Purge			93					
Toluene-d8, Surrogate Recovery, %		Heated Purge			99					
4-Bromofluorobenzene, Surrogate Recovery, %		Heated Purge								
Lab Control Sample Recovery:					Before	Added	Solvent			Limits
Allyl alcohol					ND	500	551		112.2	
Benzene					ND	50	48		95.0	
Chlorobenzene					ND	50	50		100.0	
1,1-Dichloroethene					ND	50	48		95.0	
Trichloroethene					ND	50	52		104.0	
Toluene					ND	50	50		100.0	
Dibromofluoromethane, Surrogate Recovery, %					ND	50	45		90.0	

Page QC-1

Continued on Page QC-2

Note: Any points outside control limits are denoted by an asterisk.

Environmental Chemistry, Inc.
2525 West Bellfort, Suite 175
Houston, Texas 77054

Phone 713-666-0020
Fax 713-666-41746

QUALITY CONTROL REPORT

Job: 042637

VOAM Miscellaneous Volatiles by GC/MS			Batch 20041118-LAM-1				
Type	GC/MS	Method(s)	82606 SW-846;	624EPA600	ug/L		
Lab Control Sample Recovery:							
Toluene-d8, Surrogate Recovery, %					Before	Added	Spiked
4-Bromofluorobenzene, Surrogate Recovery, %					ND	50	50
Matrix Spike Duplicates:					ND	50	50
Allyl alcohol	042639-19				MS	MSD	
Benzene	042639-19				957	1250	
Chlorobenzene	042639-19				95	99	
1,1-Dichloroethene	042639-19				97	101	
Trichloroethene	042639-19				94	100	
Toluene	042639-19				101	103	
Dibromofluoromethane, Surrogate Recovery, %	042639-19				94	98	
Toluene-d8, Surrogate Recovery, %	042639-19				48	47	
4-Bromofluorobenzene, Surrogate Recovery, %	042639-19				50	50	
Matrix Spike Recovery:					49	50	
Allyl alcohol	042639-19				Before	Added	Spiked
Benzene	042639-19				550	500	957
Chlorobenzene	042639-19				ND	100	95
1,1-Dichloroethene	042639-19				ND	100	97
Trichloroethene	042639-19				ND	100	94
Toluene	042639-19				ND	100	101
Dibromofluoromethane, Surrogate Recovery, %	042639-19				ND	100	94
Toluene-d8, Surrogate Recovery, %	042639-19				ND	50	48
4-Bromofluorobenzene, Surrogate Recovery, %	042639-19				ND	50	50
Allyl alcohol	042639-19				ND	50	49
Benzene	042639-19				550	500	1250
Chlorobenzene	042639-19				ND	100	99
1,1-Dichloroethene	042639-19				ND	100	101
Trichloroethene	042639-19				ND	100	100
Toluene	042639-19				ND	100	103
Dibromofluoromethane, Surrogate Recovery, %	042639-19				ND	100	98
Toluene-d8, Surrogate Recovery, %	042639-19				ND	50	47
4-Bromofluorobenzene, Surrogate Recovery, %	042639-19				ND	50	50
	042639-19				ND	50	50

ABC Study No. 48910

Environmental Chemistry, Inc.
2525 West Bellfort, Suite 175
Houston, Texas 77054

Phone 713-866-0020
Fax 713-866-4176

METHODS, ANALYSIS DATES, AND HOLDING TIMES

Job: 042637

Test		Sampled	Started	Completed	By/Batch	Days	Hold time
VOAM	Miscellaneous Volatiles by GC/MS by 8260B, SW-846; 624, EPA600						
	Holding time, days: 14						
042637- 1	0-Hr Control	10/26/04	11/12/04	11/13/04	LAM-2	17	Exceeded
042637- 2	0-Hr Level 1 (0.65 mg/L)	10/26/04	11/12/04	11/13/04	LAM-2	17	Exceeded
042637- 3	0-Hr Level 2 (1.3 mg/L)	10/26/04	11/12/04	11/13/04	LAM-2	17	Exceeded
042637- 4	0-Hr Level 3 (2.5 mg/L)	10/26/04	11/18/04	11/18/04	LAM-1	23	Exceeded
042637- 5	0-Hr Level 4 (5.0 mg/L)	10/26/04	11/12/04	11/13/04	LAM-2	17	Exceeded
042637- 6	0-Hr Level 5 (10 mg/L)	10/26/04	11/18/04	11/18/04	LAM-1	23	Exceeded
042637- 7	72-Hr Control Solution	10/26/04	11/12/04	11/13/04	LAM-2	14	Met
042637- 8	72-Hr Level 1 Biotic Solution (0.65 mg/L)	10/26/04	11/12/04	11/13/04	LAM-2	14	Met
042637- 9	72-Hr Level 1 ABIOTIC Solution (0.65 mg/L)	10/26/04	11/12/04	11/13/04	LAM-2	14	Met
042637- 10	72-Hr Level 2 Solution (1.3 mg/L)	10/26/04	11/12/04	11/13/04	LAM-2	14	Met
042637- 11	72-Hr Level 3 Solution (2.5 mg/L)	10/26/04	11/12/04	11/13/04	LAM-2	14	Met
042637- 12	72-Hr Level 4 Solution (5.0 mg/L)	10/26/04	11/12/04	11/13/04	LAM-2	14	Met
042637- 13	72-Hr Level 5 Solution (10 mg/L)	10/26/04	11/12/04	11/13/04	LAM-2	14	Met

December 6, 2004

Ms. Marcy Banton
Lyondell Chemical Company
One Houston Center, Suite 1600
1221 McKinney Street
Houston, Texas 77010

RE: Allyl alcohol analysis, ECI Job Number

Dear Ms. Banton:

Attached are the results for the Allyl alcohol analyses performed on the samples recently submitted. A total of 35 samples were received from ABC laboratories for analysis. The samples were received with chain of custody documentation. The samples were received in the appropriate container type and the temperature upon receipt was 2 degrees Celsius. The 14 day holding time was exceeded on 6 of the samples prior to receipt by the laboratory.

The samples were logged in upon receipt and were stored in the refrigerator until the time of analysis. All samples were analyzed within the holding time specified by the EPA for volatile organic analytes.

The samples were analyzed in accordance with EPA Method 8260. Method 8260 is a Gas chromatography/Mass Spectrometry (GC/MS) technique for analyte separation and identification. Sample introduction into the GC/MS was accomplished using the heated purge and trap Method 5030.

A calibration curve was established using seven standards of known concentration prepared at 50, 100, 200, 500, 1000, 1500 and 2000 ug/L. The initial calibration was confirmed using a calibration verification standard. The calibration verification standard was prepared from a second source than that used for preparation of the calibration standards. The calibration response met the requirements for a linear calibration as specified in Method 8260.

A Method Detection Limit (MDL) study was performed using the statistical evaluation of seven replicate analyses of a standard near the expected detection limit as described in 40 CFR, Part 136, Appendix B. The reasonableness of the detection limit was verified by analysis of a standard within two to three times the calculated MDL.

The quality control requirements of Method 8260 were met for these samples. This includes specifications for tuning of the detector, calibration verification, analysis and evaluation of method blanks, and laboratory control samples.

September 30, 2005



Lyondell Chemical Company
Page 2

Please note on the QC report that the results for matrix spike and matrix spike duplicate samples indicate that the precision and accuracy for Allyl alcohol in the sample matrix is not as good as for other 8260 compounds. ECI does not have sufficient data points to calculate statistical control limits for the matrix spike and duplicate analysis for Allyl alcohol at this time. I believe the higher variability in the Allyl alcohol analysis, as compared to other compounds, is due to its relatively low response factor. Allyl alcohol yields an average response factor of approximately 0.017; many of the other compounds typically analyzed by Method 8260 have response factors greater than or equal to 0.100. Lower response factors are typically encountered for compounds such as alcohols that do not purge very efficiently even upon heating.

Thank you for the opportunity to be of service to Lyondell Chemical Company. Please contact us if you have any questions regarding this work or require any additional assistance on our part.

Sincerely,

Carolyn Elmore
Environmental Chemistry, Inc.

ABC Study No. 48910

APPENDIX D. REPLICATE AREA UNDER THE GROWTH CURVE

Table D-1. Replicate Area Under the Growth Curve During a 72-Hour Exposure of *Pseudokirchneriella subcapitata* to Allyl Alcohol 20906MB

Nominal Concentration (mg total product/L)	Rep	Area Under the Growth Curve		
		0-24 Hr	0-48 Hr	0-72 Hr
Control	A	46	320	2000
	B	40	330	1900
	C	44	350	2100
0.65	A	44	340	2100
	B	37	340	2000
	C	46	370	2100
1.3	A	37	330	2100
	B	36	340	1900
	C	28	310	1900
2.5	A	9.6	110	830
	B	11	120	830
	C	4.8	110	900
5.0	A	-5.3	0.24	30
	B	2.4	17	64
	C	-4.0	-4.3	24
10	A	-4.0	-12	-18
	B	-5.3	-16	-20
	C	-6.7	-16	-16

Note: Values are rounded to two significant figures.

ABC Study No. 48910

APPENDIX E. REPLICATE GROWTH RATE

Table E-1. Replicate Growth Rate During a 72-Hour Static Exposure of *Pseudokirchneriella subcapitata* to Allyl Alcohol 20906MB

Nominal Concentration (mg total product/L)	Rep	Growth Rate (cells/mL/hour)		
		0-24 Hr	0-48 Hr	0-72 Hr
Control	A	0.065	0.062	0.066
	B	0.061	0.064	0.066
	C	0.064	0.065	0.067
0.65	A	0.064	0.064	0.068
	B	0.059	0.065	0.066
	C	0.065	0.066	0.067
1.3	A	0.059	0.064	0.067
	B	0.058	0.065	0.065
	C	0.050	0.064	0.066
2.5	A	0.024	0.046	0.055
	B	0.027	0.046	0.055
	C	0.014	0.047	0.056
5.0	A	-0.024	0.013	0.013
	B	0.0076	0.014	0.019
	C	-0.017	0.0055	0.016
10	A	-0.017	-0.0083	-0.0035
	B	-0.024	-0.012	0.0013
	C	-0.034	-0.0052	0.0025

Note: Values are rounded to two significant figures.